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Modern Applied Botany: Changes in the perception of applied botanists to themselves and others during the last century. Three recent examples of the scientific potential of this field.[#]

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Summary

Subsequent to a short chronicle of the history of applied research in plant biology in Germany, the relevance of modern *Applied Botany* is illustrated by three relevant post-harvest processes. The metabolic reactions that play a key role in the determination of quality of the related plant-derived commodities from each are presented. Increased understanding of the processes involved in these treatments has facilitated improvement of product quality in the resulting products. In each instance, it has been necessary to regard plant metabolism comprehensively and not to focus on a single physiological process. Moreover, the various interactions with the environment have to be considered. These illustrations demonstrate that transfer and application of basic plant knowledge into product-related research can provide significant information that is valuable for improvement of plant-derived products. In some instances, these correlations can even account for traditional and well-established processes, as illustrated for the malting process. However, interdisciplinary work and intensive cooperation with growers and producers must be an integral part of developing feasible and economically acceptable solutions that can be transferred into practice. Ultimately, the major challenge in *Applied Botany* today is the implementation of new concepts and ideas into product-related research. In consequence, modern *Applied Botany* acts as a mediator between basic plant science and industrial, product-related research.

Keywords: Applied botany; cocoa; coffee; malting; *Coffea arabica*; *Theobroma cacao*; *Hordeum vulgare*; plant-derived commodities; post-harvest treatments; product quality.

Historical notes

Within the first decades of the past century, major improvements in experimental capabilities occurred. One major result of these advancements was improved scientific knowledge of the major metabolic processes of life and metabolism. In plant science, photosynthesis was a major center of focus, but our understanding of other metabolic processes also was greatly improved. As result of these major advances, many scientists involved in basic or fundamental research began to feel that only these studies represent respectable research. As a consequence, research of colleagues involved in more applied topics, were often not considered to be of significance. This was despite the fact that their studies provided many new important insights for forestry, agriculture, and food production. In the wake of this contempt, many botanists involved in applied research felt that the German Botanical Society – *Deutsche Botanische Gesellschaft*, which was founded in 1882 and was supposed to deal with the scientific interests of all plant biologists, no longer played that role. Because of their dissatisfaction, in 1902, scientists working on applied aspects of botanical research established the Society for Applied Botany – *Vereinigung für Angewandte Botanik*. This new society gathered scientists working in forestry, horticulture, agriculture, plant protection, post-harvest management and food quality. To enhance their scientific communication, in 1919, an official journal for the new society, the *Zeitschrift für Angewandte Botanik*, was initiated. Eventually, to also make the journal accessible for non-German speakers, it was refocused as the international research organ *Journal of Applied Botany*. Due to partial overlap in fields of research, in 2004, the journal was extended by including the German Society for Quality Research in Plant Foods – *Deutsche Gesellschaft für Qualitätsforschung*. Today the *Journal of Applied Botany and Food Quality* is well established as a successful online-only and open access journal.

At present, research combining fundamental and applied science is not stigmatized – quite the contrary. Due to the fascinating possibilities offered by modern molecular tools, the alliance between fundamental or basic and applied research is omnipresent and the lines within the research of many are blurred. Increasing inclusion of university education on the one hand, and elevated public perception of research on the other, even requires such a combination. This paradigm change is also nicely reflected by changes in university studies: the traditional university education in the sense of Humboldt is substituted by bachelor and master studies in which the academic education is complemented – or even exchanged – by vocational training. Apart from scientific considerations, focus on job training aspects requires integration of applied research in modern basic science. Thus, within the last few decades, the perception of applied botanists to themselves in regard to the conception of scientists involved in fundamental research has undergone major changes. The lines between applied and fundamental research are no longer important. As a result, in 2015, the *Vereinigung für Angewandte Botanik* was abandoned as independent scientific organization and integrated as the *Sektion für Angewandte Botanik* in the *Deutsche Botanische Gesellschaft*.

Applied Botany – a broad array of actual research topics

At the time of its inception, research positioned within the *Vereinigung für Angewandte Botanik* was related to and focused on agriculture, forestry, horticulture, plant protection, post-harvest management and food quality. Although much of the research was done by well-known techniques, even at this early time, quite modern approaches in respect to plant biotechnology were studied by a number of members of the society. An intriguing example for such contemporary research is given by an article from HUGO FISCHER (1919), who outlined the usage of elevated CO_2 concentrations to enhance

[#] This treatise is dedicated to Böle Biehl and Reinhard Lieberei who passed away in January and March 2019. Both were outstanding scientists who had a strong positive impact on the entire field of Applied Botany. We greatly appreciate their innovative and successful research on post-harvest treatment of cocoa and the cultivation of other tropical crops and food plants as well as their outstanding roles as academic teachers and mentors.

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plant growth and production. Fischer's innovative work represents the basis for the modern approaches of CO_2 fertilization, a technique presently used in modern greenhouse technology.

Other than a few modern day topics, e.g., the consequences of global warming or contamination of plant derived commodities, the core areas of interest for *Applied Botany* been little altered over the last hundred years. However, the strategy – and especially the *modi operandi* – changed significantly. Two main alterations should be noted: a shift in experimental and field work, and consideration of appropriate metabolic relationships. Descriptive and empirical studies in *Applied Botany* have largely been substituted by carefully outlined experimental approaches and sound field trials. However, the approaches used are often very broad. Accordingly, the required spectrum of methods is far more diverse than in most other biological disciplines. These range from highly sophisticated analytical and phytochemical methods such as LC-MS, NMR, or biochemical studies, to techniques of modern molecular biology.

One major distinguishing feature of modern *Applied Botany* is the effective and successful implementation of research into praxis. In consequence, many research projects that are funded by various federal scientific organizations require recognition and approval of the practical relevance of the corresponding projects. Presently, such institutional funding exhibits two major advantages: first, small- and medium-sized companies that cannot afford the costs for requisite research, get access to novel insights relevant for their production. Secondly, the required fund raising does not depend on a direct sponsorship by the industry, and thus, conflicts of interest are reduced. Nonetheless, to transfer research results into praxis requires combining the expertise of plant biologists and industrial researchers. This frequently is attained by cross-functional committees associated with the project that consist of the plant biologists conducting the research and industrial representatives. In this manner, the objectives have to be defined, results have to be evaluated, and corresponding solutions must be elaborated. Such strategy, however, creates major drawbacks in the evaluation of concurrent research projects, e.g., dealing with the underlying scientific bases. Unfortunately, a research proposal that is directed toward applied content frequently still is assessed as a "knock-out criterion" for projects submitted to research foundations that mainly support fundamental or basic research. Nonetheless, apart from problems related to fund raising, research in the broad field of *Applied Botany* exhibits many advantages. In this context, the application of basic plant biology to find relevant answers and to develop problem-oriented solutions opens fascinating fields of research. To illustrate these intriguing possibilities, three interesting examples are now described.

Consideration of metabolic processes frequently is lacking in industrial research

Physiological processes influence the composition of plants and, accordingly, plant-derived commodities. As a result, these metabolic events determine product quality. As plant metabolism is significantly influenced by various exogenous factors, such as light, temperature, and water availability, metabolic processes can be – at least in part – deliberately modulated by altering these factors. Unfortunately, despite the relevance of these factors for product quality of plant-derived commodities, until the present, the related physiological processes and their regulation frequently are not adequately considered. This is obvious when appropriate cultivation and post-harvest processing techniques must be optimized or when alternative approaches need to be developed. Basic metabolic relationships often remain unseen when technologists and mechanical engineers not trained in botany dominate and predefine industrial research. Plant scientists with required plant biological expertise as well as a broad range of methods are quite rare in industrial research and development divisions. Awareness of the importance of physiological processes on product quality is frequently lacking. Apparently, the flux of knowledge from basic plant sciences to industrial applied research and *vice versa* is quite limited and must be enhanced in the future, e.g. by increasing cross-linkages and communication platforms. In this context, modern *Applied Plant Biology*, considering and focusing on fundamental metabolic processes, exhibits the potential to serve as an important mediator for transfer of basic knowledge and analytical knowhow into industrial research approaches.

Metabolic changes in tropical seeds during post-harvest treatments

All major tropical plant-derived commodities such as coffee, cocoa, or tea, are generated by various post-harvest treatments including specific drying procedures. In this context, we have to consider that the seeds of tropical plants – in contrast to the orthodox seeds of crops grown in Europe – are not dormant; but recalcitrant[1]. Accordingly, as soon as they are exposed to favorable conditions, the seeds germinate and – as a result – metabolism is released. This normally requires removal of the fruit flesh, which is involved in suppression of germination in the fruits. The timing and manner of induction of metabolic processes, which influence the composition of the seeds in major ways, strongly impacts the quality of the plant-derived products. These relationships are seen in the post-harvest processing of the two most important tropical commodities, cocoa and coffee.

Cocoa fermentation

Traditionally, post-harvest processing of cocoa beans entails fermentation in small heaps: in the course of this procedure, the sticky, sugary pulp that surrounds the cocoa or cacao seeds, is degraded anaerobically by yeasts (for review see: Amoa-Awua, 2015; Voigt and Lieberei, 2015). After some days, the major share of the pulp is gone, and the formerly compact and solid heap is interspersed by numerous cavities and caverns. As a result, the heap is aerated, inducing massive development of aerobic bacteria that oxidize the ethanol produced by the yeasts to yield acetic acid (Biehl et al., 1985; Schwan et al., 2015). Consequently, the pH of the heap decreases strongly, and, due to influx of acetic acid, the so far still vital (living) cocoa seeds are killed. In the course of the nascent decompartmentation, proteases obtain access to the storage proteins, which are hydrolysed to yield large amounts of amino acids and peptides (Voigt et al., 1994; Voigt and Lieberei, 2015). These essential aroma precursors react with reducing sugars during the roasting of the cocoa beans generating Maillard products that are characteristic for typical cocoa aroma (Afoakwa et al., 2008).

Due to tremendous increases in demand for raw cocoa, processing of that commodity has been more and more mechanized. Heap fermentation has largely been replaced by fermentation in shallow boxes, which hold batches of up to one ton of material. Unfortunately, the corresponding raw cocoa, especially that produced in Malaysia, had a very high content of acetic acid and was weak in flavour (Biehl, 1985). When considering the biological background, the solution for improving the quality was obvious: the amount of ethanol produced in the anaerobic phase of fermentation must be reduced. To achieve this goal, two main strategies were developed: bean spreading and pod storage. In the case of pod storage, the intact cocoa fruits after harvest were stored on the plantation for certain time period, usually for one week (Meyer et al., 1989). As a result, due to active metabolism within the fruits, the amount of sugars, which are fermented in the anaerobic phase of fermentation, is strongly reduced (Biehl et al., 1989). Moreover, this preconditioning of the pulp reduced the time span required to liquefy the pulp. Consequently, the liquid drains faster and aerobic conditions are attained earlier (Meyer

[1] According to Roberts (1973), recalcitrant seeds do not undergo a maturation drying and are not capable of withstanding water loss.

et al., 1989). The same relationships apply for the "bean spreading" process (BIEHL et al., 1990). However, in this case, the fruits are opened directly after harvesting, and the seeds are extracted. Instead of transferring them instantly into the fermentation boxes, the cocoa seeds are spread on drying patios for a certain time, before they are subjected to the fermentation process.
Both of these methods of pulp preconditioning, which were developed by considering the basic metabolic processes of post-harvest physiology, are now standard procedures. They significantly increase the quality of cocoas fermented in shallow boxes (AMOA-AWUA, 2015).

Green coffee processing

Dried seeds of the coffee tree (*Coffea arabica* L.), denoted as green coffee, represent one of the top ten commodities in global trade. Traditionally, in the coffee industry, green coffee had always been considered as an inanimate plant material. Consequently, all attempts to explain the characteristic differences of wet- and dry-processed green coffees were premised solely on the basic chemical and physical parameters of green coffee (SELMAR et al., 2015). The same accounts for the approaches of the coffee producers to modify the sensory properties and to increase product quality. However, inspired by the major advances in optimization of cocoa fermentation by consideration of the metabolic events occurring in that process, analogous approaches were initiated for green coffee processing. As is true for cocoa beans, green coffee also represents germinable seeds that lack dormancy. However, in contrast to the recalcitrant seeds of cocoa, coffee seeds are classified as intermediate seeds, because they can tolerate water loss without losing their germinability (ELLIS et al., 1990; RADWAN et al, 2014). Because of these similarities, induction of germination during post-harvest processing is of particular interest (SELMAR et al., 2006; BYTOF et al., 2007). In addition, in coffee seeds, as in other living cells, any decrease in water content during drying will also induce typical drought stress responses (KRAMER et al., 2010). Both metabolic syndromes, i.e., germination and stress, will change the composition of the affected cells, and thus of the green coffee seeds. Inexplicably, these relationships had not been taken into consideration before plant biologists insistently undertook research on green coffee processing. By combining classical as well as novel and innovative approaches of modern *Applied Botany*, the causes of the well-known quality differences of dry- and wet-processed green coffees could be determined: the characteristic sensory differences of wet- and dry-processed coffees could be attributed to differences in metabolic activities in coffee beans during the course of the various post-harvest treatments. As predicted, in the first phases of green coffee processing, the coffee seeds do indeed germinate (KRAMER et al., 2010). However, water availability of the seeds which are differentially processed differs greatly. Loss of water is much faster in the depulped and wet-processed seeds than in those that are still surrounded by the fruit flesh during dry processing. As a consequence, the extent and velocity of germination is greater during wet processing and the composition of the differentially-processed green coffee seeds differs. In addition, the drought stress responses also are induced differently in the wet- and dry-processed coffees. These differences in stress-induced metabolism also generate significant differences in the composition of the dried coffee beans (KRAMER et al., 2010; KLEINWÄCHTER and SELMAR, 2010), in particular in the content of γ-aminobutyric acid (BYTOF et al., 2005). As a consequence of these plant physiological insights, a paradigm change in the coffee industry was initiated. Today, it is common knowledge that green coffee represents living organisms that exhibit active metabolism. That observation provides major potential for quality improvement during processing (SELMAR and BYTOF, 2006; KLEINWÄCHTER and SELMAR, 2010). Research on green coffee processing is one of the most intriguing examples in *Applied Botany* for establishing that the knowledge and consideration of underlying basic plant physiological relationships opened new doors: deliberate alterations of the corresponding processing conditions could induce desired changes in the quality of plant-derived commodities (KLEINWÄCHTER et al., 2014). As a result, new approaches have been introduced and become established in praxis – the most intriguing one is undoubtedly the successful implementation of an additional stationary storage phase in the so-called BECOLSUB-process. BECOLSUB represents a type of green coffee processing that is in-between classical dry- and wet-processing. In analogy to wet-processing, the coffee cherries are depulped. However, the fermentation step, which is inherently responsible for the degradation of residues of the fruit flesh, is substituted by mechanical removal of the sticky pulp. Because of its economic advantages, this procedure was favored by the coffee producers. Unfortunately, the quality of the corresponding coffees was far lower than that of the wet-fermented ones. Based on biochemical insight, it was postulated

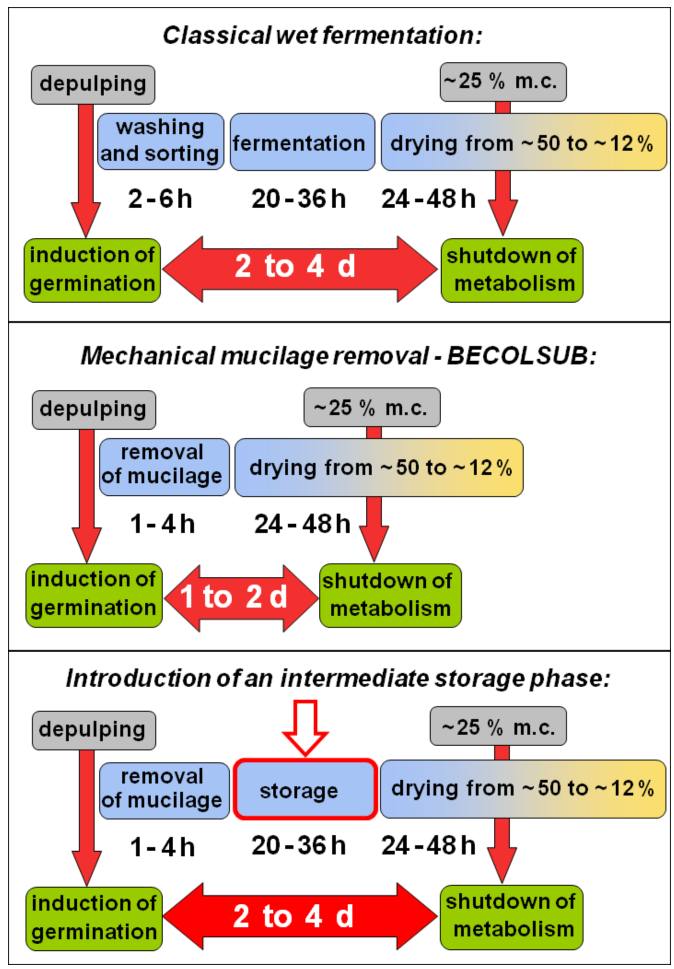

Fig. 1: Introduction of an intermediate storage phase in the BECOLSUB green coffee processing.
The removal of the pulp of coffee fruits induces seed germination. During the course of drying, the seed moisture content (m.c.) decreases from initially 50% down to 12%. When the water content falls below about 25%, the metabolic activities are shut down. Whereas the related time span of metabolic activity is about two to four days in the case of classical wet processing, due to the omission of a fermentation step, it is much shorter in the case of BECOLSUB, where mucilage is removed mechanically (for details see KLEINWÄCHTER et al., 2014). An introduction of an interim storage phase enhances this time span and thereby leading to an improvement of green coffee quality

that extension of the phase exhibiting an active metabolism will solve this problem (Selmar et al., 2005). Accordingly, an additional storage step of the mechanically cleaned beans was introduced (Fig. 1). Indeed, as predicted, when the beans were stored for one day after pulp removal, the quality of the coffees generated by BECOLSUB processing increased significantly. This interim storage phase has now been implemented worldwide by coffee producers.

Malting – an ancient biotechnological approach still lacks physiological insight

For plant biologists, it is obvious that many metabolic processes occur in living cells. Unfortunately, this basic observation is frequently disregarded with respect to its significance for post-harvest treatments or food processing. Indeed, in dormant seeds, e.g., cereals, metabolic processes are almost completely shut down. After imbibition, germination is induced and metabolic activity is regained. These coherences have been exploited for centuries in numerous malting processes. It is matter of common knowledge that germination of barley and wheat has to be induced as a precondition for starch degradation. This is necessary to produce maltose, the required product for the alcoholic fermentations by yeasts. Nonetheless, there are many additional metabolic reactions that occur in the course of germination, and, thus, during the malting process. Nonetheless, so far, these apparently simple relationships had not been considered adequately and comprehensively. In this context, an intriguing issue concerns the role and impact of carbon dioxide (CO_2). Maltsters always appraised CO_2 as toxic, although any scientific data were lacking. It is a general maltsters`s rule that "*this poisonous substance*" has to be removed efficiently from the germinating seedlings. Yet, plant physiologists consider CO_2 as an inert gas for plants. Accordingly, they explain the negative effect of this "*toxic compound*" by the lack of oxygen, which results from respiration of the seedlings in the large malting vessels. In order to clear up this apparently contrary situation and to determine the real impact of CO_2 during malting, the relevant physiological processes occurring in the barley seeds in the steep tanks was analyzed (Kleinwächter et al., 2012). As predicted, CO_2 does not reveal any toxic effects for barley seeds; but, high concentrations of this gas greatly inhibit germination, and, thus, biosynthesis of the relevant enzymes. Even more surprisingly, lack of oxygen was not responsible for this observed inhibition of germination. Indeed, the exact mode of action is still unknown, but there may be competitive interactions between oxygen and carbon dioxide (Kleinwächter et al., 2012). Based on this insight, one of the major problems in industrial-scale malting, the blocking of the filters during lautering (separation of the wort from the spent grains), can be explained. This problem is especially relevant when steeping is performed in high layers - today tanks with a height of 3-4 m are frequently used. Inhomogeneous aeration results in massive differences in the distribution of CO_2 and O_2. These corresponding spatial differences in the partial pressures of oxygen and carbon dioxide are responsible for large variation in the progression of germination, and, thus, in an asynchronous degradation of carbohydrates. In this context, the hydrolysis of galactomannans located in the cell wall is of special interest. Insufficient degradation entails the generation of gels, which in turn, cause severe problems by blocking the filters during lautering. As predicted, comprehensive analyses of the physiological processes occurring in the barley seeds during malting verified the presence of heterogeneities, and clarified the biochemical background of this phenomenon. Simple modifications in process control, e.g., changes in the extent of aeration or reduction in the height of the malt bed enable the production of malts exhibiting enhanced homogeneity (Kleinwächter et al., 2014; Müller et al., 2013). This example nicely demonstrates that even well-established processes can be optimized by transferring plant basic knowledge into industrial research.

Conclusions

The three examples outlined above confirm that metabolic processes during post-harvest treatments play a key role in the quality manifestation of plant-derived commodities. Consequently, increased understanding of the relevant processes enables us to take steps to improve product quality. However, these striking examples also underline that it is not sufficient to focus only on one single physiological process, but that it is necessary to consider comprehensively the plant metabolism and its complexity. This is especially important in studies of various interactions with the environment. Moreover, these examples demonstrate that the transfer of plant basic knowledge into product-related research should initiate strong impulses for product-related research. As noted above, this even applies to traditional and well-established processes, as is illustrated for the malting process. Continuous interdisciplinary work and collaboration in project accompanying committees assists development of feasible and economically acceptable solutions that can be transferred directly into practice.

Apart from consideration of the basic scientific approaches, implementation of new concepts and ideas into product-related research is a great challenge. In this context, a major future goal is to optimize the related transfer of knowledge. For this reason, modern *Applied Botany* has to act as a mediator between basic plant science and industrial, product-related research.

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